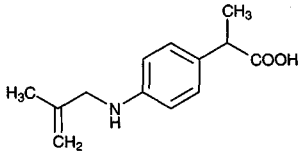
Alminoprofen



Molecular formula: $C_{13}H_{17}NO_2$
Molecular weight: 219.28
CAS Registry No.: 39718-89-3
Merck Index: 308

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 50 µL 200 µg/mL glafenic acid in mobile phase + 100 µL 17 mM acetic acid + 7 mL diethyl ether, vortex for 10 s, centrifuge at 2000 g at 3° for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum, reconstitute the residue in 200 µL mobile phase, inject a 50 µL aliquot.

HPLC VARIABLES

Column: 300 × 4.6 10 µm µBondapak C18
Mobile phase: MeOH:water 50:50 with 1% glacial acetic acid
Flow rate: 0.85
Injection volume: 50
Detector: UV 235

CHROMATOGRAM

Retention time: 8
Internal standard: glafenic acid (5.6)
Limit of detection: 1000 ng/mL

KEY WORDS

plasma

REFERENCE

Paillet,M.; Merdjan,H.; Brouard,A.; Doucet,D.; Barreteau,H.; Fredj,G. Rapid determination of alminoprofen in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 455–459.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 253

CHROMATOGRAM

Retention time: 4.69
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, synovial fluid

Sample preparation: 1 mL Plasma + 100 μL saturated NaCl solution + 100 μL 17 mM acetic acid + ketoprofen + 7 mL diethyl ether, shake centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air with mild heating, reconstitute the residue in 500 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeOH:water:acetic acid:DMSO 50:46:0.8:3
Flow rate: 1
Injection volume: 20
Detector: UV 251

CHROMATOGRAM
Retention time: 8.1
Internal standard: ketoprofen (11.7)
Limit of quantitation: 2 μg/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Tod,M.; Pobel,C.; Le Gros,V.; Louchahi,K.; Petitjean,O.; Brion,N.; Garcia-Macé,J.L. A population pharmacokinetic study of alminoprofen penetration into synovial fluid, *Biopharm.Drug Dispos.*, **1995**, *16*, 627–634.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 201.7

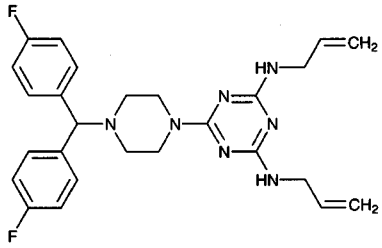
CHROMATOGRAM
Retention time: 18.695

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Almitrine

Molecular formula: $C_{26}H_{29}F_2N_7$
Molecular weight: 477.56
CAS Registry No.: 27469-53-0, 29608-49-9 (dimethanosulfonate)
Merck Index: 309



SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 25.905

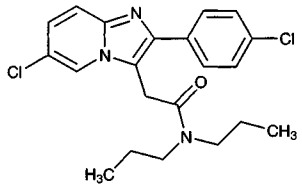
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Alpidem



Molecular formula: $C_{21}H_{23}Cl_2N_3O$
Molecular weight: 404.34
CAS Registry No.: 82626-01-5
Merck Index: 318

SAMPLE

Matrix: blood
Sample preparation: 2 mL Plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently on a horizontal agitator for 10 min, centrifuge at 2800 g for 10 min. Remove the organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot. (Buffer was saturated ammonium chloride, diluted 25% with water, adjusted to pH 9.5 with 25% diluted ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 10 mM KH_2PO_4 adjusted to pH 2.6 with orthophosphoric acid. At the end of the day wash column with water at 0.8 mL/min for 1 h and MeOH at 0.8 mL/min for 1 h.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 200
Detector: UV 247

CHROMATOGRAM

Retention time: 10.97
Limit of detection: 19.2 ng/mL

OTHER SUBSTANCES

Extracted: zopiclone, zolpidem, suriclone
Simultaneous: p-nitrophenol, ketotifen, tiaprofenic acid, vincristine, sultopride, pyrimethamine, nimodipine

KEY WORDS

plasma

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. High-performance liquid chromatographic assay with diode-array detection for toxicological screening of zopiclone, zolpidem, suriclone and alpidem in human plasma, *J.Chromatogr.*, **1993**, *616*, 95–103.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 20 μL 2.5 μg/mL IS in MeOH, vortex, centrifuge at 11000 g for 4 min, inject a 200 μL aliquot onto column A with mobile phase A, elute with mobile phase A for 2 min, elute the contents of column A onto column B with mobile phase B for 1.5 min, remove column A from the circuit and elute column B with mobile phase B, monitor the effluent from column B. Clean column A by back-flushing with MeCN: water 50:50, MeCN, MeOH:water 50:50, and water (all at 2 mL/min).

HPLC VARIABLES

Column: A 75 × 2.1 30-40 μm Perisorb C18; B 20 × 4.6 40 μm Pelliguard LC8 + 150 × 4.6 5 μm Hypersil BDS C8

Mobile phase: A water; B MeCN:MeOH:25 mM KH_2PO_4 40:15:45
Flow rate: 1.5
Injection volume: 200
Detector: F ex 255 em 423

CHROMATOGRAM
Retention time: 14.4
Internal standard: 6-chloro-2-(3,4-dimethoxyphenyl)-N,N-dipropylimidazo[1,2-a]pyridine-3-acetamide (SL 80.0633) (8)
Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: trazodone, zolpidem
Noninterfering: diazepam, nordiazepam, nitrazepam, lorazepam, cimetidine, amitriptyline, clomipramine, ranitidine

KEY WORDS
plasma; column-switching

REFERENCE
Flaminio,L.; Ripamonti,M.; Ascalone,V. Determination of alpidem, and imidazopyridine anxiolytic, and its metabolites by column-switching high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.A*, **1994**, *668*, 403–411.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 247

CHROMATOGRAM
Retention time: 10.97
Limit of detection: <120 ng/mL

KEY WORDS
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine;

pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, tissue

Sample preparation: Homogenize brain tissue with 5 volumes water. 200-500 µL Plasma or brain homogenate + 1 mL 1 M pH 10 carbonate buffer + 20 µL 10 µg/mL IS in MeCN + 7 mL diethyl ether, agitate on a Bioblock REAX 3 agitator for 20 min, centrifuge at 1000 g at 4° for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 120 µL MeCN:water 40:60, inject a 90 µL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 µm Spherisorb ODS
Mobile phase: MeCN:MeOH:10 mM pH 2.5 KH_2PO_4 50:10:40
Flow rate: 1
Injection volume: 90
Detector: F ex 257 em 398

CHROMATOGRAM

Internal standard: 6-chloro-2-(3,4-dimethoxyphenyl)-N,N-dipropyl-imidazo[1,2-a]pyridine-3-acetamide (SL 80.0633)
Limit of detection: 1 ng/mL

KEY WORDS

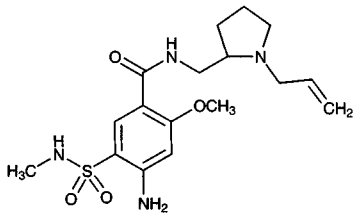
plasma; rat; brain

REFERENCE

Garrigou-Gadenne,D.; Durand,A.; Thenot,J.P.; Morselli,P.L. The disposition and pharmacokinetics of alpidem, a new anxiolytic, in the rat, *Drug Metab.Dispos.*, **1991**, *19*, 574–579.

Alpiropride

Molecular formula: $C_{17}H_{26}N_4O_4S$
Molecular weight: 382.48
CAS Registry No.: 81982-32-3
Merck Index: 319



SAMPLE

Matrix: blood, urine

Sample preparation: Plasma. Condition a 3 mL C8 Analytichem SPE cartridge with 1 volume (2.7 mL) MeOH and 1 volume buffer A, do not allow to dry. Mix 1 mL plasma + 100 µL 10 µg/mL amisulpride in 10 mM HCl + 1 mL buffer A, add to SPE cartridge, rinse the sample container with 1 mL buffer A and add the rinse to the SPE cartridge, wash with 1 volume water, wash with 2 mL buffer B, dry the column for 1 min, wash with 200 µL acetone, dry for 30 s, elute with 1 mL buffer C, add 50 µL buffer D, evaporate to dryness under a stream of air, reconstitute in 200 µL mobile phase, sonicate for 1 min, inject an aliquot. Urine. Connect a Baker 3 mL ion exchange quaternary aminesilicane-bonded silica gel SPE cartridge on top of a 3 mL Baker carboxylic acid-bonded silica gel SPE cartridge, condition with 1 volume (2.7 mL) buffer D, 1 volume of water, 1 volume of MeOH, and 1 volume of water. Mix 1 mL urine + 100 µL 10 µg/mL amisulpride in 10 mM HCl + 1 mL water, add to SPE cartridges, rinse the sample container with 2 mL water and add the rinse to the SPE cartridges, wash with 1 mL water, remove the top column, wash the bottom column with 1 volume of water and 2 volumes of MeOH, dry the column for 1 min, elute with 1 mL buffer D, evaporate the eluate to dryness under a stream of air at 45°, reconstitute in 200 µL mobile phase, sonicate for 1 min, inject an aliquot. (Buffer A was 10 mL triethylamine in 1 L water, pH adjusted to 7.00 with acetic acid. Buffer B was MeOH:water 20:80. Buffer C was 10 mL triethylamine + 7 mL acetic acid in 1 L MeOH. Buffer D was 2.10 mL concentrated HCl in 250 mL MeOH (100 mM).)

HPLC VARIABLES

Guard column: 10 cm long Chrompack reverse-phase pellicular material
Column: 250 × 4.6 10 µm LiChrosorb RP-8
Mobile phase: MeCN:MeOH:buffer 160:80:760 (Buffer was 10 mL triethylamine + 760 mL water adjusted to pH 6.8 with acetic acid (about 4.2 mL).)
Flow rate: 2
Injection volume: 175
Detector: UV 230

CHROMATOGRAM

Retention time: 6.0
Internal standard: amisulpride (4.9)

OTHER SUBSTANCES

Simultaneous: metoclopramide, alizapride, aspirin, theophylline, acetaminophen, caffeine, isosorbide-5-mononitrate, acenocoumarol, carbamazepine, nitrazepam, clonazepam, codeine, nitrofurantoin
Noninterfering: indomethacin, orphenadrine, furosemide, cisplatin, amitriptyline, isosorbide dinitrate, propranolol

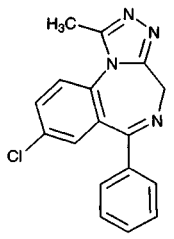
KEY WORDS

plasma; SPE; alpiropride is IS

REFERENCE

de Jong,A.P.; Wittebrood,A.J.; du Châtinier,W.M.; Bron,J. Liquid chromatographic analysis of alizapride and metoclopramide in human plasma and urine using solid-phase extraction, *J.Chromatogr.*, **1987**, *419*, 233–242.

Alprazolam



Molecular formula: $C_{17}H_{13}ClN_4$
Molecular weight: 308.77
CAS Registry No.: 28981-97-7
Merck Index: 320
Lednicer No.: 3 197

SAMPLE

Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. Mix 1 mL plasma or serum with 200 μL 512 nM IS in MeOH:water 5:95, add to the SPE cartridge, wash with 2 mL water, wash with 50 μL MeOH. Elute with 200 μL and 100 μL MeOH, evaporate the eluate to dryness under a stream of air at 37°, reconstitute the residue with 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 4 μm Novapak C18
Mobile phase: MeCN:MeOH:10 mM pH 3.7 K_2HPO_4 30:2:100
Flow rate: 1.5
Injection volume: 20
Detector: UV 240

CHROMATOGRAM

Retention time: 8.5
Internal standard: flunitrazepam (9.8)
Limit of detection: 5 nM

OTHER SUBSTANCES

Extracted: clonazepam, nitrazepam
Simultaneous: amobarbital, carbamazepine, citalopram, clobazam, clozapine, diazepam, doxepin, ethosuximide, norclobazam, oxazepam, oxcarbamazepine, pentobarbital, phenobarbital, phenytoin, primidone, valproic acid, zopiclone
Interfering: medazepam, midazolam, nordiazepam, temazepam

KEY WORDS

SPE; plasma; serum

REFERENCE

Åkerman,K.K.; Jolkkonen,J.; Parviainen,M.; Penttilä,I. Analysis of low-dose benzodiazepines by HPLC with automated solid-phase extraction, *Clin.Chem.*, **1996**, *42*, 1412–1416.

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeCN and 5 mL water. Mix 1 mL plasma with 10 μL 1 μg/mL IS in MeOH, dilute with 5 mL 1 M NaCl, mix briefly. Add to the SPE cartridge, wash with 10 mL water, elute with 5 mL MeCN:water 20:80, evaporate the eluate to dryness under reduced pressure at 60°, dissolve the residue in 50 μL MeOH and 100 μL mobile phase, inject an aliquot.; SPE

HPLC VARIABLES

Column: 150 × 4.6 5 μm Develosil C8-5 (Nomura Chemical, Seto, Japan)
Mobile phase: MeCN:0.5% pH 4.5 KH_2PO_4 30:70
Flow rate: 1
Detector: UV 230

CHROMATOGRAM
Retention time: 17.5
Internal standard: estazolam (14.3)
Limit of detection: 500 pg/mL

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Yasui,N.; Otani,K.; Kaneko,S.; Ohkubo,T.; Osanai,T.; Sugawara,K.; Chiba,K.; Ishizaki,T. A kinetic and dynamic study of oral alprazolam with and without erythromycin in humans: In vivo evidence for the involvement of CYP3A4 in alprazolam metabolism, *Clin.Pharmacol.Ther.*, **1996**, *59*, 514–519.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

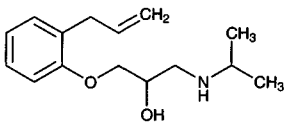
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 220.5

CHROMATOGRAM
Retention time: 16.972

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Alprenolol



Molecular formula: $C_{15}H_{23}NO_2$
Molecular weight: 249.35
CAS Registry No.: 13655-52-2, 13707-88-5 (HCl)
Merck Index: 321
Lednicer No.: 1 177

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 270

CHROMATOGRAM

Retention time: 6.18
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol;

aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH, dilute with mobile phase.

HPLC VARIABLES

Column: 150 × 3.9 Novapak-phenyl-4
Mobile phase: MeOH:15 mM pH 6.5 sodium acetate buffer 81:19
Flow rate: 1.2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 3.2-3.4

OTHER SUBSTANCES

Simultaneous: trifluoperazine, triflupromazine

REFERENCE

Al-Obaid,A.M.; Hagga,M.E.M.; El-Khawad,I.E.; El-Mahi,O.H.M. Simultaneous quantitation of some phenothiazine drug substances and their monosulphoxide degrades by high performance liquid chromatography (HPLC), *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1369–1389.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Brownlee Newguard guard column
Column: 100 × 4.6 5 μm Hypercarb
Mobile phase: MeOH containing 20 mM NaOH
Flow rate: 1
Injection volume: 40
Detector: UV 275

CHROMATOGRAM

Retention time: 9.2

OTHER SUBSTANCES

Simultaneous: related substances

REFERENCE

Karlsson,A.; Berglin,M.; Charron,C. Robustness of the chromatographic separation of alprenolol and related substances using a silica-based stationary phase and selective retention of metoprolol and related substances on a porous graphitic carbon stationary phase, *J.Chromatogr.A*, **1998**, *797*, 75–82.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 7 μm RP-8 guard column
Column: 125 × 4.0 5 μm Hibar LiChrosorb RP-8
Mobile phase: MeCN:pH 3.0 phosphate buffer containing 2 mM sodium *n*-octylsulfonate 29:71
Flow rate: 1
Injection volume: 40
Detector: UV 275

CHROMATOGRAM

Retention time: 8.0

OTHER SUBSTANCES

Simultaneous: related substances

REFERENCE

Karlsson,A.; Berglin,M.; Charron,C. Robustness of the chromatographic separation of alprenolol and related substances using a silica-based stationary phase and selective retention of metoprolol and related substances on a porous graphitic carbon stationary phase, *J.Chromatogr.A*, **1998**, *797*, 75–82.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.0

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyram-

ide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 8.28

OTHER SUBSTANCES

Also analyzed: acebutolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, tymazoline, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-

10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 62:38:0.1
Column temperature: 40
Flow rate: 1
Detector: F ex 460 em 550

CHROMATOGRAM

Retention time: 12.4, 15.9 (enantiomers)
Limit of detection: 31-39 fmole

OTHER SUBSTANCES

Also analyzed: oxprenolol, propranolol

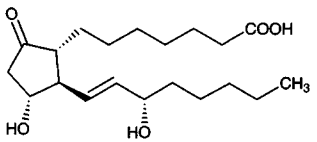
KEY WORDS

derivatization; chiral

REFERENCE

Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

Alprostadil



Molecular formula: $C_{20}H_{34}O_5$
Molecular weight: 354.49
CAS Registry No.: 745-65-3
Merck Index: 8063
Lednicer No.: 3 2

SAMPLE

Matrix: blood

Sample preparation: Condition a Bond Elut Certify C18 SPE cartridge with water, MeCN, and 20 mM citric acid. Add 1 mL plasma to SPE cartridge, wash with 1 mL 20 mM citric acid, wash with 2 mL MeOH:water 10:90, wash with 2 mL cyclohexane, elute with 3 mL 3% ammonia in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 500 μL MeCN, add 200 μL 10 mM DBD-PZ in MeCN, add 300 μL 10 mM 2,2'-dipyridyl disulfide and 10 mM triphenylphosphine in MeCN, let stand at room temperature for 30 min, inject an aliquot. (DBD-PZ prepared from 123 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN added dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, extract three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water, dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-(1-piperazinyl)-2,1,3-benzoxadiazole (DBD-PZ) as orange crystals, mp 121-2° (J. Chromatogr. 1991, 588, 61).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: Gradient. MeCN:water from 35:65 to 60:40 over 1 h
Column temperature: 40
Flow rate: 1
Detector: F ex 440 em 569

CHROMATOGRAM

Retention time: 29.9
Limit of detection: 1.7-5 fmole

OTHER SUBSTANCES

Extracted: dinoprost (prostaglandin F2α), dinoprostone (prostaglandin E2), limaprost, 6-ketoprostaglandin F1α, prostaglandin F1α, prostaglandin D2, prostaglandin A1, prostaglandin B1

KEY WORDS

plasma; rat; SPE

REFERENCE

Toyo'oka,T.; Ishibashi,M.; Terao,T.; Imai,K. Sensitive fluorometric detection of prostaglandins by high performance liquid chromatography after precolumn labelling with 4-(*N,N*-dimethylaminosulphonyl)-7-(1-piperazinyl)-2,1,3-benzoxadiazole (DBD-PZ), *Biomed.Chromatogr.*, **1992**, *6*, 143–148.

SAMPLE

Matrix: blood

Sample preparation: 10 μL Serum + 44 μL MeOH + 1 μL pyridine, sonicate for 5 min, add 25 μL 100 mM reagent in DMF, add 20 μL 400 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in MeOH, let stand at 25° for 2 h, centrifuge, inject an aliquot. (Reagent

was 2-(5-hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole which was synthesized as follows. Pass dry hydrogen chloride into a mixture of 12.6 g methyl 2-furoate, 4.5 g paraformaldehyde, and 3.4 g anhydrous zinc chloride in 50 mL dry chloroform for 3 h while holding the reaction temperature at 30°. After cooling pour the contents of the flask into 100 mL cold water, remove the chloroform layer, extract the aqueous layer with chloroform (cf Coll. Czech. Chem. Commun. 1960, 25, 1058). Combine the chloroform layers, neutralize, dry over anhydrous calcium chloride, evaporate, distil to give 5-chloromethyl furyl-2-carboxylic acid methyl ester (bp 108°/4 mm Hg). Reflux 10 g 5-chloromethyl furyl-2-carboxylic acid methyl ester and 25 g silver carbonate in 100 mL THF: water 70:30 for 5 h, filter through Celite, concentrate the filtrate under reduced pressure, chromatograph the product on silica gel with chloroform to give 5-hydroxymethyl furyl-2-carboxylic acid methyl ester as a light yellow oil. Add a solution of 2.9 g 5-hydroxymethyl furyl-2-carboxylic acid methyl ester in 30 mL dichloromethane to 12 g pyridinium chlorochromate in 100 mL, dichloromethane, stir at room temperature for 4 h, evaporate to dryness under reduced pressure, chromatograph on silica with dichloromethane to give 5-formyl furyl-2-carboxylic acid methyl ester as a light yellow powder. Add 10 mL concentrated nitric acid dropwise to 20 g 4-bromoveratrole in 60 mL acetic acid while keeping the temperature at 10-30° with occasional cooling, when the addition is complete pour the reaction mixture into ice-water. Collect the precipitate and dissolve it in 500 mL hot EtOH, add activated charcoal, filter, add 40 mL water to the filtrate to give 4,5-dimethoxy-2-nitrobromobenzene as a light yellow crystalline solid (mp 121-122°). Prepare sodium sulfide by melting together 5 g sodium sulfide nonahydrate and 700 mg sulfur, add this mixture to 5 g 4,5-dimethoxy-2-nitrobromobenzene in 50 mL EtOH:water 95:5, reflux for 30 min, pour into ice-water, collect the solid, recrystallize from dichloromethane to give di(4,5-dimethoxy-2- nitrophenyl)sulfide as yellow needles (mp 231-232°). Add 15 mL concentrated HCl dropwise to 1.5 g di(4,5-dimethoxy-2-nitrophenyl)sulfide and 4.5 g tin powder stirred at 40-50° in 150 mL EtOH, reflux for 1 h, cool to room temperature, filter, add 1.17 g 5-formyl furyl-2-carboxylic acid methyl ester to the filtrate, reflux for 1 h, cool, filter, chromatograph the solid on silica gel with dichloromethane, recrystallize from EtOH to give 5-(5',6'-dimethoxybenzothiazolyl)-N-furan-2-carboxylic acid methyl ester as a yellow powder (mp 192-202°). Add 2 mL hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen and may expplode when distilled in air!) to 800 mg 5-(5',6'-dimethoxybenzothiazolyl)-N-furan-2-carboxylic acid methyl ester in 20 mL EtOH, reflux for 30 min, collect the solid, wash with MeOH, dry under vacuum over phosphorus pentoxide to give 2-(5-hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole as a light yellow solid (mp 226-228°).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil-II 5C18 HG
Mobile phase: Gradient. MeCN:water from 70:30 to 75:25 over 25 min, to 100:0 over 15 min, maintain at 100:0.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 363 em 452

CHROMATOGRAM

Retention time: 51
Limit of detection: 50 fmole

OTHER SUBSTANCES

Extracted: arachidonic acid, dinoprost, dinoprostone, lauric acid, linoleic acid, linolenic acid, margaric acid, myristic acid, myristoleic acid, oleic acid, palmitic acid, palmitoleic acid, prostaglandin $F_{1\alpha}$, stearic acid

KEY WORDS

derivatization

REFERENCE

Saito,M.; Ushijima,T.; Sasamoto,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-furyl)-5,6-dimethoxybenzothiazole as a precolumn fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography and its application to the assay of fatty acids in human serum, *Anal.Sci.*, **1995**, *11*, 103–107.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 5-50 mg compound in 1-2 mL MeCN, add a 3-fold molar excess p-nitrophenacyl bromide, add a 2-fold molar excess of N,N-diisopropylethylamine, let stand at room temperature for 15 min, dilute with 50 mL ethyl acetate, wash with 25 mL 200 mM pH 2.30 citrate buffer, wash with 200 mM pH 7.80 phosphate buffer, wash with 25 mL water, dry the organic layer over anhydrous sodium sulfate, evaporate to dryness under reduced pressure at 45°, prepare a solution in dichloromethane:chloroform 50:50, inject an aliquot.

HPLC VARIABLES

Column: two 250 × 2.1 Zorbax-Sil columns in series
Mobile phase: Dichloromethane:MeCN:DMF 80:20:0.5
Flow rate: 0.28
Detector: UV 254

CHROMATOGRAM

Retention time: 34.25

OTHER SUBSTANCES

Simultaneous: dinoprostone

KEY WORDS

derivatization; normal phase

REFERENCE

Morozowich,W.; Douglas,S.L. Resolution of prostaglandin p-nitrophenacyl esters by liquid chromatography and conditions for rapid, quantitative p-nitrophenacylation, *Prostaglandins*, **1975**, *10*, 19–40.

SAMPLE

Matrix: bulk

Sample preparation: Add 10 mg prostaglandin to 1 mL 15 mg/mL 2-bromo-4'-nitroacetophenone in MeCN, add 5 μL N,N-diisopropylethylamine, mix, let stand at room temperature for at least 2 h. Evaporate to dryness under a stream of nitrogen, reconstitute the residue in 1 mL chloroform, add 500 μL 200 mg/mL silver nitrate in water, mix thoroughly, centrifuge. Filter (0.2 μm) the chloroform layer and inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 250 × 4.6 10 μm Partisil SCX impregnated with silver ion (Prepare the column by pumping 80 mL 1 M silver nitrate in water through the column, wash the column with water until a negative test for silver ion is obtained, wash with 50 mL EtOH, wash with 50 mL acetone, wash with 50 mL ethyl acetate, wash with 50 mL trichloroethane, and wash with 50 mL hexane.)
Mobile phase: Dioxane:MeCN 99.94:0.06 (Caution! Dioxane is a carcinogen!)
Detector: UV 254

CHROMATOGRAM

Retention time: k' 1.2

OTHER SUBSTANCES

Simultaneous: degradation products, dinoprost, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS
derivatization

REFERENCE
Merritt,M.V.; Bronson,G.E. High-performance liquid chromatography of p-nitrophenacyl esters of selected prostaglandins on silver ion-loaded microparticulate cation-exchange resin, *Anal.Biochem.*, **1977**, *80*, 392–400.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Prepare a 500 μg/mL solution of the bulk drug in EtOH. Evaporate a 2 mL aliquot of the EtOH solution or an aliquot of the formulation containing 1 mg compound to dryness under a stream of nitrogen, add 200 μL 20 mg/mL α-bromoacetonaphthone, swirl, add 100 μL 10 μL/mL N,N-diisopropylethylamine, swirl, heat at 45° for 1 h with swirling every 15 min, evaporate to dryness under a stream of nitrogen, reconstitute with 10 mL 400 μg/mL methylprednisolone in dichloromethane, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μPorasil
Mobile phase: Dichloromethane:1,3-butanediol:water 99.5:0.5:0.05
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 15
Internal standard: methylprednisolone (25)

OTHER SUBSTANCES
Simultaneous: dinoprostone, 8-isoprostaglandin E_1, 8-isoprostaglandin E_2, 5,6-trans-prostaglandin E_2

KEY WORDS
derivatization; injections; normal phase

REFERENCE
Zoutendam,P.H.; Bowman,P.B.; Ryan,T.M.; Rumph,J.L. Quantitative determination of alprostadil (PGE_1) in bulk drug and pharmaceutical formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *283*, 273–280.

SAMPLE
Matrix: enzyme incubations
Sample preparation: Add 500 μL enzyme incubation to 1 mL MeOH, mix, add 4 mL 100 mM citric acid, add 500 mg anhydrous sodium sulfate, extract twice (alprostadil, dinoprostone) or 3 times (dinoprost) with 5 mL portions of dichloromethane. Pass the extracts through 1 g anhydrous sodium sulfate and evaporate them to dryness, reconstitute with 1 mL anhydrous MeCN containing a 3-fold molar excess of α,p-dibromoacetophenone, add 2 μL diisopropylethylamine, let stand for 1 h, evaporate to dryness, reconstitute with 200 μL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:water 50:50
Flow rate: 1.2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Limit of quantitation: 5 μM

OTHER SUBSTANCES
Extracted: metabolites, dinoprost, dinoprostone

KEY WORDS
derivatization

REFERENCE
Fitzpatrick,F.A. High-performance liquid chromatographic analysis of prostaglandins formed during in vitro incubations with prostaglandin 15-dehydrogenase, *J.Pharm.Sci.*, **1976**, *65*, 1609–1613.

SAMPLE
Matrix: formulations
Sample preparation: 100-300 mg Gel ointment + 3 mL MeOH, mix vigorously, filter (0.2 μm). Evaporate 2 mL to dryness under a stream of nitrogen, reconstitute the residue in mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:20 mM pH 4.9 KH_2PO_4 40:60
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Extracted: prostaglandin E1-α-cyclodextrin, prostaglandin A1, prostaglandin B1

KEY WORDS
ointment

REFERENCE
Yamamura,K.; Yamada,J.-I.; Yotsuyanagi,T. High-performance liquid chromatographic assay of antiinflammatory drugs incorporated in gel ointments. Separation and stability testing, *J.Chromatogr.*, **1985**, *331*, 383–388.

SAMPLE
Matrix: seminal fluid
Sample preparation: 1-5 μL Seminal fluid + 100 μL 5 μM IS in MeOH, mix, add 3 mL water to the supernatant, acidify to pH 3-4 with 100 mM HCl, extract with 7 mL ethyl acetate. Remove the ethyl acetate layer and evaporate it to dryness, reconstitute with MeOH. Evaporate to dryness in a clean tube, add 10 mg finely-powdered potassium bicarbonate:sodium sulfate 50:50, add 50 μL 0.4-1 mM 4-bromomethyl-7-acetoxycoumarin in acetone, add 50 μL 200 μM dibenzo-18-crown-6 in acetone, heat in the dark at 80° for 1 h, cool, inject a 20-40 μL aliquot. (Prepare 4-bromomethyl-7-acetoxycoumarin as follows. Reflux 50 g 7-hydroxy-4-methylcoumarin (β-methylumbelliferone) and 100 mL acetic anhydride for 1 h, cool, pour into 500 mL cold water, filter, dry the solid, recrystallize from EtOH to give 4-methyl-7-acetoxycoumarin. Reflux 10 g 4-methyl-7-acetoxycoumarin, 9 g N-bromosuccinimide, a little 2,2'-(azobis(2-methylpropionitrile) (α,α'-azobisisobutyronitrile, Eastman), and 100 mL carbon tetrachloride for 20 h, cool, evaporate under reduced pressure to remove the solvent, wash the residue with water, filter, dry, recrystallize from ethyl acetate/cyclohexane to give 4-bromomethyl-7-acetoxycoumarin (mp 184-185°) (J. Chromatogr. 1982, 234, 121).)

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrosorb RP-18
Mobile phase: Gradient. MeCN:water from 30:70 to 90:10 over 99 min (Concave 1 curve (64 min) using a Japan Spectroscopic Model GP-A30 solvent programmer).
Column temperature: 50
Flow rate: 1
Injection volume: 20-40
Detector: F ex 365 em 460 following post-column reaction. The effluent from the column mixed with 100 mM NaOH pumped at 0.4 mL/min and the mixture flowed through a 10 m × 0.5 mm ID stainless steel coil at 50° to the detector. (The prostaglandins are chromatographed as the coumarin derivatives then hydrolyzed in the post-column reactor to fluorescent 7-hydroxy-4-hydroxymethylcoumarin.)

CHROMATOGRAM
Retention time: 47
Internal standard: 16-methylprostaglandin $F_{1\alpha}$ (49)
Limit of detection: 10 fmole

OTHER SUBSTANCES
Extracted: dinoprost, dinoprostone

KEY WORDS
derivatization; post-column reaction

REFERENCE
Tsuchiya,H.; Hayashi,T.; Naruse,H.; Takagi,N. Sensitive high-performance liquid chromatographic method for prostaglandins using a fluorescence reagent, 4-bromomethyl-7-acetoxycoumarin, *J.Chromatogr.*, **1982**, *231*, 247–254.

SAMPLE
Matrix: seminal fluid
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 20 mL EtOH and 20 mL water. Dilute seminal fluid to 1 mL, adjust to pH 3.5 with aqueous formic acid, centrifuge, add the supernatant to the SPE cartridge, wash with 20 mL EtOH:water 15:85, wash with 20 mL water, remove excess water mechanically, wash with 20 mL hexane, elute with 4 mL methyl formate. Dry the eluate under a stream of nitrogen, add 10-20 μL reagent, vortex for 1 min, let stand at room temperature for 8 min, add 90-180 μL water, extract with an equal volume of ethyl acetate, centrifuge at 2000 g for 3 min, evaporate the organic layer to dryness under a stream of nitrogen, reconstitute, inject an aliquot (J. Chromatogr. 1985, 349, 431). (Prepare the reagent by stirring 100 mg pyridinium dichromate in 50 mL MeCN at room temperature for 1 h, centrifuge, use the supernatant (5 mM; 1.9 mg/mL), store at 5°, discard after 2 days (J. Chromatogr. 1983, 282, 435).); SPE

HPLC VARIABLES
Column: 220 × 2.1 5 μm Spheri-5 C18
Mobile phase: Gradient. MeCN:0.5 mM formic acid 30:70 for 4.5 min, to 40:60 (step gradient).
Flow rate: 0.4
Injection volume: 1
Detector: UV 229

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Extracted: dinoprostone, 19-hydroxyprostaglandin E_1, 19-hydroxyprostaglandin E_2, oxoprostaglandin E_1, oxoprostaglandin E_2

KEY WORDS
derivatization; SPE

REFERENCE
Doehl,J.; Greibrokk,T. Determination of prostaglandins in human seminal fluid by solid-phase extraction, pyridinium dichromate derivatization and high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *529*, 21–32.

SAMPLE
Matrix: seminal fluid
Sample preparation: Mix 50 μL seminal fluid with 500 μL dilute HCl (pH 3.0) and 500 μL ethyl acetate, vortex. Remove the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 200 μL water, add to a Toyopak-ODS SPE cartridge, elute with 200 μL MeOH. 100 μL Eluate + 100 μL 100 mM 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in water + 100 μL 1% aqueous pyridine + 100 μL 15 mM 2-(5-hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran in DMF, heat at 37° for 1 h, inject a 10 μL aliquot. (Synthesis of 2-(5-hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran is as follows. Slowly add 153 g freshly distilled phosphorus oxychloride to 73 g anhydrous DMF with stirring at room temperature, add 125 g sesamol in portions over 4 h, stir at room temperature overnight, pour into ice water, filter. Dissolve the solid in ether and wash with water, dry over anhydrous magnesium sulfate, evaporate to dryness, recrystallize from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as slightly-yellow crystals (mp 125-126°). Pass HCl gas into 15.6 g ethyl 2-thiophenecarboxylate, 4.5 g paraformaldehyde, and 3.4 g zinc chloride in 50 mL chloroform with stirring at 30° over 4 h. Pour into ice water and extract with 50 mL chloroform. Wash the chloroform layer 3 times with water, wash twice with aqueous sodium bicarbonate solution, dry over anhydrous sodium sulfate, evaporate to remove the solvent, distil at 86-94°/0.15 mm Hg to yield ethyl 5-chloromethyl thiophene-2-carboxylate as a colorless oil. Heat 3 g 2-hydroxy-4,5-methylenedioxybenzaldehyde, 3.68 g ethyl 5-chloromethyl thiophene-2-carboxylate, and 2.49 g potassium carbonate in 100 mL anhydrous DMF at 110° for 16 h, filter, evaporate the filtrate to dryness under reduced pressure, chromatograph the residue on silica gel with chloroform, recrystallize from chloroform:hexane 25:75 to give 2-(5-ethoxycarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran as yellow crystals (mp 124-126°). Heat 1.5 g 2-(5-ethoxycarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran and 1.2 g hydrazine hydrate in 15 mL DMF at 70° for 1 h (Caution! Hydrazine hydrate is a carcinogen and explodes on distillation in air!), add 10 g hydrazine hydrate, add 20 mL water, filter. Wash the solid with MeOH and dry it under reduced pressure to give 2-(5-hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran as a yellow powder (mp 262-263°).)

HPLC VARIABLES
Column: 250 × 4 5 μm Wakosil ODS-II 5C18 HG
Mobile phase: MeCN:water 34:66
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 373 em 483

CHROMATOGRAM
Retention time: 125
Limit of detection: 0.1 pmole

OTHER SUBSTANCES
Extracted: dinoprost, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS
derivatization; SPE

REFERENCE
Saito,M.; Ushijima,T.; Sasamoto,K.; Yakata,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-thienyl)-5,6-methylenedioxybenzofuran and 2-(5-hydrazinocarbonyl-2-furyl)-5,6-methylenedioxybenzofuran as novel fluorescence derivatization reagents for carboxylic acids in liquid chromatography, *Anal.Chim.Acta*, **1995**, *300*, 243–251.

SAMPLE
Matrix: solutions
Sample preparation: Prepare methyl ester by treatment with excess ethereal diazomethane for 5 min, remove excess reagent under a stream of nitrogen. Dissolve 10 μg methyl ester in 200 μL anhydrous pyridine containing a 10-fold molar excess of p-nitrobenzylhydroxylamine hydrochloride, heat at 40° for 2 h, evaporate to dryness under a stream of nitrogen, reconstitute with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 600 mm long μBondapak C18
Mobile phase: MeCN:water 85:15
Flow rate: 0.75
Detector: UV 254

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: dinoprostone, prostaglandin A1, prostaglandin A2, prostaglandin B1, prostaglandin B2

KEY WORDS
derivatization

REFERENCE
Fitzpatrick,F.A.; Wynalda,M.A.; Kalser,D.G. Oximes for high-performance liquid and electron capture gas chromatography of prostaglandins and thromboxanes, *Anal.Chem.*, **1977**, *49*, 1032–1035.

SAMPLE
Matrix: solutions
Sample preparation: Dry solution under a stream of nitrogen, add 10 equivalents of reagent, vortex for 1 min, let stand at room temperature for 8 min, add a volume of water equivalent to one tenth the volume of the reaction mixture, inject a 5 μL aliquot. (Prepare the reagent by stirring 100 mg pyridinium dichromate in 50 mL MeCN at room temperature for 1 h, centrifuge, use the supernatant (5 mM; 1.9 mg/mL), store at 5°, discard after 2 days.)

HPLC VARIABLES
Guard column: 50 × 4.6 40 μm pellicular C18 (Supelco)
Column: 200 × 4.6 5 μm RP-18 (Brownlee)
Mobile phase: Gradient. MeCN:10 mM formic acid from 40:60 to 60:40 over 10 min
Flow rate: 1.5
Injection volume: 5
Detector: UV 228 for 10 min then UV 298

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 30-80 pmole

OTHER SUBSTANCES
Simultaneous: dinoprostone, prostaglandin A_1, prostaglandin A_2, prostaglandin B_1, prostaglandin B_2

KEY WORDS
derivatization

REFERENCE
Dohl,J.; Greibrokk,T. High-performance liquid chromatographic separation and ultraviolet detection of prostaglandins, oxidized by pyridinium dichromate, *J.Chromatogr.*, **1983**, *282*, 435–442.

SAMPLE
Matrix: solutions
Sample preparation: Dry solution under a stream of nitrogen, add 10-20 μL reagent, vortex for 1 min, let stand at room temperature for 8 min, add 90-180 μL water, inject a 1 μL aliquot. Alternatively, extract with an equal volume of ethyl acetate, centrifuge at 2000 g for 3 min, evaporate the organic layer to dryness under a stream of nitrogen, reconstitute, inject an aliquot. (Prepare the reagent by stirring 100 mg pyridinium dichromate in 50 mL MeCN at room temperature for 1 h, centrifuge, use the supernatant (5 mM; 1.9 mg/mL), store at 5°, discard after 2 days.)

HPLC VARIABLES
Column: 250 × 1.3 8 μm C18 (Chrompack)
Mobile phase: MeCN:10 mM pH 2.7 phosphoric acid 38:62
Flow rate: 0.06
Injection volume: 1
Detector: UV 229

CHROMATOGRAM
Retention time: 28
Limit of detection: 0.14 pmole

OTHER SUBSTANCES
Simultaneous: dinoprost, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS
derivatization; microbore

REFERENCE
Doehl,J.; Greibrokk,T. High-performance liquid chromatographic separation and determination of prostaglandins, oxidized by pyridinium dichromate. Optimization and applications, *J.Chromatogr.*, **1985**, *349*, 431–438.

SAMPLE
Matrix: solutions
Sample preparation: Mix an aliquot of a solution in MeOH with 50 μL purified 9-anthryldiazomethane reagent, after 6 h inject an aliquot. (Purify 9-anthryldiazomethane on a 500 × 7.2 7 μm PG-pak C polystyrene gel column with ethyl acetate at 1 mL/min and UV 350 detection, inject 1 mg, collect the effluent when the purified compound elutes (20-22 min) and use it within 6 h.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm PG-Pak B silica gel
Mobile phase: Gradient. Isooctane:ethyl acetate:EtOH:acetic acid 90:10:0:1 for 15 min then 80:15:4:2 for 20 min (step gradient).
Flow rate: 1.2
Detector: F ex 365 em 412

CHROMATOGRAM
Retention time: 32.5
Limit of detection: 100 pg

OTHER SUBSTANCES
Simultaneous: dinoprost, dinoprostone, HHT, hydroxyeicosatetraenoic acid, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin D_2, prostaglandin $F_{1\alpha}$, thromboxane B_2

KEY WORDS
derivatization; normal phase

REFERENCE
Yamauchi,Y.; Tomita,T.; Senda,M.; Hirai,A.; Terano,T.; Tamura,Y.; Yoshida,S. High-performance liquid chromatographic analysis of arachidonic acid metabolites by pre-column derivatization using 9-anthryldiazomethane, *J.Chromatogr.*, **1986**, *357*, 199–205.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.5 5 μm cyano (IBM)
Mobile phase: Gradient. Hexane:isopropanol 98:2 for 12 min, then to 80:20 over 10 min, maintain at 80:20
Flow rate: 1.5
Injection volume: 100
Detector: UV 214

CHROMATOGRAM
Retention time: k' 10.54

OTHER SUBSTANCES
Extracted: arachidonic acid, prostaglandin H2, dinoprostone (prostaglandin E2), prostaglandin D2, dinoprost (prostaglandin F2α), prostaglandin F1α, 6-ketoprostaglandin E1, 6-ketoprostaglandin F1α, thromboxane B2

REFERENCE
Zulak,I.M.; Puttemans,M.L.; Schilling,A.B.; Hall,E.R.; Venton,D.L. A fast, nondestructive purification scheme for prostaglandin H2 using a nonaqueous, bonded-phase high-performance liquid chromatography system, *Anal.Biochem.*, **1986**, *154*, 152–161.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve compound in 1 mL MeCN:THF 80:20, add 70 μg panacyl bromide, add 1.025 μL N,N-diisopropylethylamine, mix, let stand at room temperature for 3 h, inject an aliquot onto column A (pre-equilibrated with 10 mL dichloromethane) and elute to waste with 15 mL dichloromethane, elute the contents of column A onto column B with the mobile phase and start the gradient, monitor the effluent from column B. (Synthesize panacyl bromide (p-(9-anthroyloxy)phenacyl bromide) as follows. Add 3.04 g benzyltrimethylammonium dichloroiodate to a solution of 500 mg 4'-hydroxyacetophenone in 50 mL dichloroethane and 20 mL MeOH, reflux for 10 h, remove the solvent by distillation, add 20 mL 5% sodium bisulfite to the residue, extract four times with 40 mL portions of ether, dry over anhydrous magnesium sulfate, evaporate to dryness under reduced pressure to give p-hydroxyphenacyl chloride (mp 151-152°) (Synthesis 1988, 545). Purify p-hydroxyphenacyl chloride by suspending 100 g in 1 L boiling toluene, filter, cool to obtain white crystals of p-hydroxyphenacyl chloride. Repeat this process a number of times to obtain more pure product. Reflux 10 g 9-anthracenecarboxylic acid in 150 mL redistilled thionyl chloride for 2 h, evaporate to dryness under reduced pressure at 30°, dissolve the residue in 150 mL dry toluene containing 11.5 g p-hydroxyphenacyl chloride, reflux for 2 h, evaporate to dryness under reduced pressure, recrystallize from 200 mL hot MeCN to give p-(9-anthroyloxy)phenacyl chloride as deep yellow crystals (mp 159.8-161.6°). Dissolve 2.5 g p-(9-anthroyloxy)phenacyl chloride in 25 mL THF:MeCN 20:80,

add 8 g anhydrous LiBr, reflux briefly, cool to room temperature, filter, wash the solid with water to obtain p-(9-anthroyloxy)phenacyl bromide as deep yellow crystals (mp 173.3-173.6°).)

HPLC VARIABLES

Column: A Guard-Pak silica; B 250 × 4.6 5 μm Hibar Silica (Merck)
Mobile phase: Gradient. A was hexane:dichloromethane:THF:MeCN:MeOH 35:50:11:4:0.25. B was dichloromethane:MeOH 98:2. C was dichloromethane:MeOH:THF 92:7:1. A:B:C 100:0:0 for 35 min, to 0:100:0 over 10 min, maintain at 0:100:0 for 20 min, to 0:0:100 over 20 min, maintain at 0:0:100 for 15 min
Flow rate: 1
Injection volume: 20
Detector: F ex 253 em 445

CHROMATOGRAM

Retention time: 71
Limit of detection: 30 pg

OTHER SUBSTANCES

Simultaneous: 13,14-dihydro-15-ketoprostaglandin E_2, dinoprost, dinoprostone, 11-epi-prostaglandin E_2, 8-isoprostaglandin E_2, 6-ketoprostaglandin $F_{1\alpha}$, prostaglandin A_2, prostaglandin D_2, thromboxane B_2

KEY WORDS

derivatization; column-switching; normal phase

REFERENCE

Salari,H.; Yeung,M.; Douglas,S.; Morozowich,W. Detection of prostaglandins by high-performance liquid chromatography after conversion to *p*-(9-anthroyloxy)phenacyl esters, *Anal.Biochem.*, **1987**, *165*, 220–229.

SAMPLE

Matrix: solutions
Sample preparation: Mix 100 μL of a 0.01-10 μg/mL solution in MeOH with 100 μL 1 mg/mL 1-pyrenyldiazomethane in ethyl acetate, let stand at room temperature for 1.5 h, inject a 5 μL aliquot. (Synthesis of 1-pyrenyldiazomethane is as follows. Suspend 5 g 1-pyrenecarboxaldehyde in 80 mL EtOH, add 3.4 g hydrazine monohydrate (Caution! Hydrazine monohydrate is a carcinogen!), stir at room temperature for 3 h, filter off the product and wash it with 50 mL cold EtOH, recrystallize from EtOH to obtain 1-pyrenecarboxaldehyde hydrazone as yellow crystals (mp 186-194° d). Add 6.55 g activated manganese dioxide to 2 g 1-pyrenecarboxaldehyde hydrazone in 300 mL diethyl ether, sonicate at room temperature for about 80 min (monitor by HPLC), filter, wash the solid with a little ether, evaporate the filtrate to obtain 1-pyrenyldiazomethane as red crystals. Prepare activated manganese dioxide as follows. Stir a solution of 20 g potassium permanganate in 250 mL water at room temperature, add 10 g activated carbon (Nuchar C-190 or C-190N), stir for 16 h, filter (Buchner funnel), wash 4 times with 50 mL portions of water, dry in air, dry in an oven at 105-110° for 8-24 h (J.Org.Chem. 1970, 35, 3971). 1-Pyrenyldiazomethane is also available from Molecular Probes, Eugene OR.)

HPLC VARIABLES

Column: 150 × 4 5 μm TSK-GEL-120A ODS (TOSOH)
Mobile phase: MeCN:water 75:25
Flow rate: 1
Injection volume: 5
Detector: F ex 340 em 395

CHROMATOGRAM

Retention time: 14

Limit of detection: 20-30 fmole

OTHER SUBSTANCES

Simultaneous: dinoprost, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS

derivatization

REFERENCE

Nimura,N.; Kinoshita,T.; Yoshida,T.; Uetake,A.; Nakai,C. 1-Pyrenyldiazomethane as a fluorescent labeling reagent for liquid chromatographic determination of carboxylic acids, *Anal.Chem.*, **1988**, *60*, 2067–2070.

SAMPLE

Matrix: solutions

Sample preparation: Mix 200 μL of a 10 μM solution in DMF containing 140 mM diethylphosphorocyanidate with 200 μL 10 mM DBD-PZ in MeCN, let stand at room temperature for 6 h, inject a 1 μL aliquot. (Synthesis of 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole (DBD-PZ) is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR). Add 123 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, wash three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water,

dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole as orange crystals (mp 121-2°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:water 45:55
Column temperature: 40
Flow rate: 1
Injection volume: 1
Detector: F ex 437 em 561

CHROMATOGRAM

Retention time: 15
Limit of detection: 14 fmol

OTHER SUBSTANCES

Simultaneous: dinoprost, hydrocortisone succinate, prednisolone succinate

KEY WORDS

SPE

REFERENCE

Toyo'oka,T.; Ishibashi,M.; Takeda,Y.; Nakashima,K.; Akiyama,S.; Uzu,S.; Imai,K. Precolumn fluorescence tagging reagent for carboxylic acids in high-performance liquid chromatography: 4-substituted-7-aminoalkylamino-2,1,3-benzoxadiazoles, *J.Chromatogr.*, **1991**, *588*, 61–71.

SAMPLE

Matrix: solutions

Sample preparation: Mix 100 μL of a 100 μM solution of the carboxylic acid in water with 100 μL 100 mM 1-(3-methylaminopropyl)-3-ethylcarbodiimide in water, 100 μL 1% pyridine in water, and 100 μL 15 mM 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran in DMF, heat at 37° for 1 h, inject a 10 μL aliquot. (Synthesis of 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran is as follows. Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Buehler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Add 2 mL phosphorus oxychloride dropwise to a solution of 2 g sesamol in 3 mL DMF at 0°, heat on a steam bath with frequent shaking for 1 h, cool in ice, add 50 mL saturated sodium acetate solution, heat on a steam bath for 30 min, cool, filter, recrystallize the solid from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as colorless needles (mp 125-126°) (Bull. Chem. Soc. Jpn. 1962, 35, 1321). Stir 1.4 g ethyl 2-chloromethyl-5-oxazolecarboxylate, 1.5 g 2-hydroxy-4,5-methylenedioxybenzaldehyde, 2 g potassium carbonate, and 50 mL anhydrous DMF at 120° overnight, cool, filter. Evaporate the filtrate to dryness under reduced pressure to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 186°) (yield 39%). Reflux 260 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran, 100 mg KOH, 20 mL EtOH, and 30 mL water for 2 h, concentrate under reduced pressure, dissolve the residue in 100 mL water, wash with ethyl acetate, treat the aqueous layer with activated carbon, acidify the aqueous layer to pH 2 with 2 M HCl. Filter the precipitate and recrystallize it from EtOH to give 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 294-295°). Reflux 150 mg 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran and 5 mL thionyl chloride for 2 h, pour the reaction mixture into 300 mL petroleum ether. Filter the precipitate and

dry it over KOH to give 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (mp 290°) (Anal. Sci. 1989, 5, 525). 2-(5-Chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran is also available from Dojindo, Kumamoto, Japan. Add 2 mL hydrazine hydrate to a stirred solution of 2 g 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran in 20 mL anhydrous DMF (Caution! Hydrazine hydrate is a carcinogen!), stir at room temperature for 4 h, add 20 mL benzene (Caution! Benzene is a carcinogen!). Collect the precipitate and wash it with water and MeCN, recrystallize from DMF:benzene 50:50 to give 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as an off-white crystalline solid (mp >220° d).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil ODS-II, WS-II 5C18 HG
Mobile phase: MeCN:water 30:70
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 350 em 450

CHROMATOGRAM

Retention time: 76
Limit of detection: 0.1 pmole

OTHER SUBSTANCES

Simultaneous: dinoprost, dinoprostone, prostaglandin $F_{1\alpha}$

KEY WORDS

derivatization

REFERENCE

Saito,M.; Chiyoda,Y.; Ushijima,T.; Sasamoto,K.; Ohkura,Y. 2-(5-Hydrazinocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as a fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography, *Anal.Sci.*, **1994**, *10*, 679–681.

SAMPLE

Matrix: solutions
Sample preparation: 100 μL 10 mM compound in MeOH + 100 μL 1% pyridine in MeOH + 100 μL 15 mM reagent in DMSO, 100 μL 100 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide in MeOH, heat at 37° for 1 h, inject a 10 μL aliquot. (Reagent was 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole which was synthesized as follows. Add 10 mL concentrated nitric acid dropwise to 20 g 4-bromoveratrole in 60 mL acetic acid while keeping the temperature at 10-30° with occasional cooling, when the addition is complete pour the reaction mixture into ice-water. Collect the precipitate and dissolve it in 500 mL hot EtOH, add activated charcoal, filter, add 40 mL water to the filtrate to give 4,5-dimethoxy-2-nitrobromobenzene as a light yellow crystalline solid (mp 121-122°). Prepare sodium sulfide by melting together 5 g sodium sulfide nonahydrate and 700 mg sulfur, add this mixture to 5 g 4,5-dimethoxy-2-nitrobromobenzene in 50 mL EtOH:water 95:5, reflux for 30 min, pour into ice-water, collect the solid, recrystallize from dichloromethane to give di(4,5-dimethoxy-2-nitrophenyl)sulfide as yellow needles (mp 231-232°) (Anal. Sci. 1995, 11, 103). Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Buehler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Reflux 5.0 g ethyl 2-chloromethyl-5-oxazolecarboxylate and 11.7 g NaI in 80 mL acetone for 1 h, partition the

reaction mixture between ethyl acetate and water. Wash the organic layer with water and dry it over anhydrous sodium sulfate, evaporate to give ethyl 2-iodomethyl-5-oxazolecarboxylate as a reddish-brown oil. Reflux 7.4 g ethyl 2-iodomethyl-5-oxazolecarboxylate and 21.5 g silver carbonate in 100 mL THF:water 70:30 for 4 h, filter through Celite, evaporate under reduced pressure, chromatograph on silica gel using benzene:ethyl acetate 95:5 to give ethyl 2-hydroxymethyl-5-oxazolecarboxylate (mp 60.5-62°). Stir 2.04 g oxalyl chloride in 15 mL dichloromethane at -50° under nitrogen, add 1.54 g DMSO in 3 mL dichloromethane, after 5 min add 1.4 g ethyl 2-hydroxymethyl-5-oxazolecarboxylate in 6 mL dichloromethane, stir for 15 min at -50°, add 5.7 mL triethylamine, allow to warm to room temperature, dilute with dichloromethane, wash with water, dry over anhydrous sodium sulfate, concentrate under reduced pressure, chromatograph on silica gel using benzene:ethyl acetate 95:5 to give ethyl 2-carboxaldehyde-5-oxazolecarboxylate (mp 71.5-73°). Add 11.3 mL concentrated HCl to 750 mg di(4,5-dimethoxy-2-nitrophenyl)sulfide stirred in 100 mL EtOH, add 3.3 g tin powder at 40-45°, stir for 1 h at 40-45°, dilute with 100 mL water, pass hydrogen sulfide gas through this solution (Caution! Hydrogen sulfide is highly toxic!), filter, concentrate the filtrate under reduced pressure to give 4,5-dimethoxy-2-aminothiophenol. Take up this compound in 30 mL EtOH:acetic acid 2:1 and add 750 mg ethyl 2-carboxaldehyde-5-oxazolecarboxylate, reflux for 1 h, collect the precipitate and recrystallize it from EtOH to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as yellow needles (mp 200-201°). Add 381 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole to 20 mL EtOH containing 3 mL DMF and 5 mL hydrazine hydrate, reflux for 1 h, collect the precipitate and wash it with EtOH, dry under vacuum to give 2-(5-hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as a yellow powder (mp 255.5-280° (d)).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Wakosil-II 5C18 HG
Mobile phase: Gradient. MeCN:water from 70:30 to 100:0 over 20 min, maintain at 100:0.
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 369 em 451

CHROMATOGRAM

Retention time: 38.5

OTHER SUBSTANCES

Simultaneous: dinoprost, dinoprostone, prostaglandin F1α

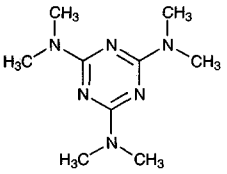
KEY WORDS

derivatization

REFERENCE

Saito,M.; Ushijima,T.; Sasamoto,K.; Ohkura,Y.; Ueno,K. 2-(5-Hydrazinocarbonyl-2-oxazolyl)-5,6-dimethoxybenzothiazole as a precolumn fluorescence derivatization reagent for carboxylic acids in high-performance liquid chromatography and its application to the assay of fatty acids in human serum, *J.Chromatogr.B*, **1995**, *674*, 167–175.

Altretamine



Molecular formula: $C_9H_{18}N_6$
Molecular weight: 210.28
CAS Registry No.: 645-05-6
Merck Index: 328

SAMPLE

Matrix: blood
Sample preparation: Cool serum or plasma and MeCN to 4°. Slowly add 600 μL MeCN to 200 μL serum or plasma, vortex for 10 s, filter (Sartorius RC4 syringe filter), inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 mm long Alltech C18
Column: 250 × 4.6 5 μm Spherisorb C18
Mobile phase: MeOH:buffer 70:30 (Buffer was 3.14 g $NaH_2PO_4.12H_2O$ and 0.108 g $Na_2HPO_4.2H_2O$ in 1 L water, pH 8.2.)
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM

Retention time: 8.5
Limit of quantitation: 150 ng/mL

OTHER SUBSTANCES

Extracted: pentamethylmelamine, 2,2,4,6-tetramethylmelamine, metabolites

KEY WORDS

serum; plasma; pharmacokinetics

REFERENCE

Barker,I.K.; Crawford,S.M.; Fell,A.F. Determination of altretamine in human plasma with high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *660*, 121–126.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 229.9

CHROMATOGRAM
Retention time: 17.833

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cells
Sample preparation: Add 20 μL 33% silver nitrate solution to a suspension of 2×10^6 cells, agitate for 10 s, sonicate for 20 min (Bransonic 52, Vel, Belgium), add 140 μL MeCN, vortex for 5 min, cool at 4° for 30 min, centrifuge at 10000 g for 30 s, add 200 μL 200 mM pH 3 phosphate buffer, inject 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 7 μm Hibar LiChrocart RP 18 (Merck)
Mobile phase: MeCN:buffer 35:65 (Buffer was 200 mM KH_2PO_4 containing 0.2% triethylamine, adjusted to pH 3.0 with 200 mM orthophosphoric acid.)
Flow rate: 1
Injection volume: 50
Detector: UV 237

CHROMATOGRAM
Retention time: 6.2
Internal standard: altretamine
Limit of detection: 2 pmol
Limit of quantitation: 8 pmol

OTHER SUBSTANCES
Extracted: daunorubicin, doxorubicin, verapamil, vincristine, S 9788

KEY WORDS
human; cells; epidermoid carcinoma; altretamine is IS

REFERENCE
Tassin,J.P.; Dubois,J.; Atassi,G.; Hanocq,M. Simultaneous determination of cytotoxic (adriamycin, vincristine) and modulator of resistance (verapamil, S 9788) drugs in human cells by high-performance liquid chromatography and ultraviolet detection, *J.Chromatogr.B*, **1997**, *691*, 449–456.